REMARKS

Claims 1 and 27-45 are pending in the case. Claims 27-29 and 43-44 are cancelled. Claims 1, 35, and 40 are amended. Specifically:

Claims 1, 35, and 40 are amended to remove reference to cyanobacteria. These claims are further amended to incorporate the language of several dependent claims defining the amino acid used for substitution as "a naturally occurring or synthetic amino acid selected from the group consisting of tryptophan, isoleucine, leucine, phenylalanine, and derivatives thereof". This amendment is supported by cancelled claims 27-29 and 43-44.

Claims 1, 35, and 40 are amended to make the language of the claims more clear by indicating the oxygen-resistant iron hydrogenase is derived from an oxygen-sensitive iron hydrogenase. Claim 35 is further amended to address the antecedent basis of "oxygen-sensitive iron hydrogenase" in the last line of the claim. This amendment is supported by Applicants' claims as filed.

Claim 40 is amended to make the language of the claim more clear by changing "oxygen-resistant" to "oxygen sensitive" in the third line of the claim. This amendment corrects an obvious error.

Other amendments are made to the claims to correct obvious typographical errors. Applicants believe that no new material has been added.

All references to the specification in this amendment refer to Application Publication No. 2006/0228774. Applicants respectfully request reconsideration of the application as follows:

I. Claim Rejections under 35 U.S.C. § 112, Second Paragraph

Claims 1 and 27-45 are rejected as indefinite and vague in the recitation of the phrase "identified amino acid residues" which the Examiner considers ambiguous and confusing. Applicants' specification describes the identified amino acid residues as those that project into the H_2-channel interior of the target hydrogenase. See, for example, paragraphs [0045] and [0064].

Claims 40-45 are rejected as indefinite and vague for the recitation of "a derivative of an oxygen-sensitive iron hydrogenase" and the use of the term "derivative" in claim 40 as the Examiner indicates it is unclear whether a structural derivative lacking functional activity is included. Applicants believe claim 40 as amended addresses the Examiner's concerns.

Claims 40-45 are rejected as indefinite and vague for reciting "an oxygen-resistant iron hydrogenase from green algae or cyanobacteria... wherein one or more residues in the oxygen-resistant hydrogenase are substituted..." which the Examiner considers confusing as to the scope of the iron hydrogenase. Applicants believe claim 40 as amended addresses the Examiner's concerns.

Claims 1 and 27-34 are rejected as indefinite as "the oxygen-sensitive iron hydrogenase" lacks antecedent basis. Applicants believe claim 1 as amended addresses the Examiner's concerns.

Claims 36-39 are rejected as indefinite for reciting proteins outside the scope of claim 35. Applicants believe claim 35 as amended addresses the Examiner's concerns.

Claims 27, 33, and 38 are rejected as indefinite for reciting "synthetic or derivatized amino acid" which the Examiner believes is confusing in the aspect of substituting such an amino acid for a naturally occurring amino acid. It is well known to one of skill in the art that a polypeptide can be produced using chemical methods such as direct peptide synthesis using solid-phase techniques. Protein synthesis can be performed manually or by automated synthesis, for example, Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer) is an automated peptide synthesis system. These techniques permit incorporation of synthetic or derivatized amino acids into a polypeptide.

Claims 43-45 are rejected as indefinite for reciting the phrase "wherein one or more residues that line the hydrogen channel of the oxygen-sensitive hydrogenase are substituted". Applicants believe amended claim 40 addresses the Examiner's concerns.

Claims 1 and 27-34 are rejected as indefinite for reciting "An oxygen-resistant iron hydrogenase derived from a green algae or a cyanobyacteria by substitution". Applicants believe amended claim 1 addresses the Examiner's concerns.

II. Claim Rejections under 35 U.S.C. § 112, First Paragraph

A. Written Description Rejection

Claims 1 and 27-45 are rejected for failing to comply with the written description requirement. The Examiner asserts the claims lack enough structural features of any or all iron hydrogenase proteins among the group of green algae or cyanobacteria having hydrogenase activity. The Examiner further asserts that when substantial variation exists within the genus, sufficient structure and variety of species must be described to reflect the variation within the

genus. While Applicants respectfully disagree with the Examiner's assertions, in order to advance prosecution, claims 1, 35, and 40 are amended to remove any reference to cyanobacteria, thus mooting the rejection as to that aspect of the claims.

Written description requirements for a claimed genus can be satisfied by actual reduction to practice of a representative number of species, reduction to drawings, by disclosure of relevant, identifying characteristics such as structural, physical, and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics.

Reduction to practice: Applicants have reduced to practice the ability to construct oxygen-resistant hydrogenases using computer modeling to identify H_2-channel residues suitable for substitution by an amino acid having properties that limit O_2 diffusion through the channel, then generating host cells transformed with such oxygen-resistant hydrogenases. Applicants demonstrate their invention in green algae, having produced two oxygen-resistant mutants with V240W substitutions capable of increased production of H_2. *See* Examples 1-3.

Reduction to drawings: Figure 1A shows a PILEUP/GENEDOC protein alignment of 5 iron hydrogenases from *Clostridium*, *Desulfovibrio*, and *Chlaydomonas*. The sequence alignments demonstrate a high overall sequence identity and even greater sequence identity between the residues forming the channel. See paragraphs [0042] and [0043]. By demonstrating sequence alignments with the 5 iron hydrogenases and providing desirable sequence identities, Applicants provide sufficient description relating to how iron hydrogenases from a variety of species can be subjected to the same procedures. Figure 1B contains homology models of HydA1 and HydA1 superimposed on CpI, and demonstrates the ability to identify H_2-channels and active sites. The catalytic core region of CpI is aligned with the protein sequence of HydA1 in Figure 2, and contains a number of similar and identical residues found in the HydA1 sequence indicating the catalytic core region of HydA1. Figures 3 and 4 show predicted H_2-channel structures in wild-type and mutant HydA1 and demonstrate the narrowing of the H_2-channel after substitution of specified amino acids with bulky amino acids. Figure 5 demonstrates that the HydA1 cDNA genomic insert having the V240W mutation was present in the transformed *C. reinhardtii*. Figures 6 and 7 show activity of oxygen-resistant hydrogenases in the presence of various levels of oxygen, demonstrating that the mutants are capable of hydrogen production in the presence of oxygen as predicted. Thus, the figures reveal the versatility of Applicants' creation through the use of the protocols used to generate the data.

Disclosure of structural, physical, and/or chemical properties: Applicants describe the structural properties of all iron-hydrogenases as having three distinct motifs containing highly conserved residues. See paragraph [0042]. Applicants provide the sequences of several H_2-channels as well as the sequence of each motif. The motifs contain a series of cysteine residues shown to have functional activity within the catalytic center. Further, Applicants provide in Figures 1B, 3, and 4 structural models of the wild-type and mutated H_2-channels. These models depict the structural change when specified amino acids are substituted with amino acids capable of narrowing the channel diameter. Applicants also include data indicating the mutated iron hydrogenases have an increased ability to produce hydrogen in the presence of oxygen relative to wild-type iron hydrogenases. See Example 3.

Coupling of functional characteristics with a known or disclosed correlation between function and structure: Use of sequence alignments and computer modeling eliminates the guesswork in predicting the effect of substituting one amino acid for another. First, using sequence alignments between the HydA1 wild-type protein and CpI allow the inventor to identify essential domains, *i.e.* the active site and the H_2-channel making up the core region of iron hydrogenases. See paragraph [0062]. Second, comparison of the structural models permits identification of critical residues important in affecting channel diameter. Once the inventors selected the candidate residues, computer modeling allowed visualization of the effect of substituting the candidate amino acids with amino acids having properties that limit oxygen diffusion through the channel while allowing hydrogen diffusion out of the channel. Applicants provide the data validating the correlation between narrowing of the channel and resistance to the inhibitory effects of oxygen on hydrogenase activity. See Example 3.

Substantial variation within the genus: Applicants describe an iron hydrogenase as an enzyme having three highly conserved motifs with conserved cysteine residues - these motifs should share at least 90% homology with a known hydrogenase such as CpI. See paragraph [0043]. Thus, iron hydrogenase variation within the green algae genus is actually quite small.

Therefore, for any one of the above reasons, Applicants' claims are sufficiently supported by the written description.

B. Enablement Rejection

Claims 1 and 27-45 are rejected as lacking enablement. Specifically, the Examiner maintains the claims are not enabled as to any iron hydrogenase from any green algae as well as any cyanobacteria derived by substituting one or more identified amino acid residues within the

hydrogen channel with any or all amino acid residues. Further, the Examiner asserts the claims must enumerate the identified amino acids and point out those amino acids having properties which limit oxygen diffusion through the channel.

While Applicants respectfully disagree with the Examiner's assertions, in order to expedite prosecution claims 1, 35, and 40 are amended to remove any reference to cyanobacteria and to specify those amino acids capable of limiting oxygen diffusion through the channel, thus mooting the rejection as to these aspects of the claims.

As mentioned in prior communications, the relevant factors considered when determining whether undue experimentation is required include, but are not limited to: (a) the breadth of the claims; (b) the nature of the invention; (c) the state of the prior art; (d) the level of one of ordinary skill; (e) the level of predictability in the art; (f) the amount of direction provided by the inventor; (g) the existence of working examples; and (h) the quantity of experimentation needed to make or use the invention based on the content of the disclosure (*In re Wands*, 858 F.2d at 737). Those factors reiterated by the Examiner's are discussed below.

The breadth of the claims: The Examiner asserts the scope of the claims is not commensurate with the enablement with respect to the "extremely large number iron hydrogenase including many mutants and variants broadly encompassed by the claims". Applicants' claims as amended now indicate the iron hydrogenase is from green algae. Green algae iron hydrogenases are enabled by the specification at, for example, Figure 1A which displays the iron hydrogenase sequence alignments for three bacteria (two clostridia, one desulfovibrio) and green algae, Figure 1B which shows a green algae hydrogenase structural model superimposed with a clostridium hydrogenase model, Figure 2 which displays the sequence alignment of a clostridium hydrogenase core sequence with a green algae protein, paragraph [0036] which indicates the sequence identity for iron hydrogenase family proteins is at least 66%, paragraph [0037] which teaches the structural characteristics of all iron hydrogenases, paragraph [0042] which describes the conserved motifs identified in all iron hydrogenase family members, and Examples 1-3 which apply these teachings and demonstrate the production of a green algae oxygen-resistant iron hydrogenase.

Level of predictablility: The Examiner asserts that since the amino acid sequence of a protein determines its structural and functional properties, knowledge of those amino acids in a protein sequence which are tolerant of modification and those which are conserved in order to obtain the desired activity are required. Applicants go to great efforts describing and

demonstrating how to mitigate the level of unpredictability. For example, Applicants identify the residues lining the H_2-channel by comparing a HydA1 sequence to a known iron-hydrogenase, CpI. See Figures 1-4. The conserved residues are seen in these same figures, and used in conjunction with computer modeling to determine which residues if substituted would most affect channel diameter. Example 1 demonstrates the use of computer modeling to provide approximate HydA1 structure and H_2-channel environment. When a selected amino acid is replaced with a bulky amino acid, the modeling software predicts the effects on the channel environment and diameter. See Figures 2 and 3, and Tables 1 and 2, as well as paragraphs [0063]-[0067]. Further, the Examples demonstrate Applicants' knowledge in the way the substitution of an amino acid residue lining the H_2-channel affects channel diameter (structure) and hydrogen production in the presence of oxygen (function). Thus, the level of predictability is such that one of skill in the art would be capable of making and using oxygen-resistant hydrogenases within the scope of the claims.

Amount of direction provided by Applicants: Applicants teach that an unknown hydrogenase is first compared to a known hydrogenase to identify the H_2-channel and conserved regions. This procedure is well known to one skilled in the art and requires sequencing the unknown hydrogenase and using readily available software programs to perform sequence alignments with the known hydrogenase. See, for example, Figures 1-2 and paragraph [0062]. Applicants then teach the use of computer modeling to identify those residues projecting into the H_2-channel as candidates for substitution with a bulky amino acid. See, for example, paragraph [0063]. Applicants describe application of *in silico* testing to determine the potential of a particular substitution to reduce channel diameter. See, for example, paragraphs [0064] and [0065]. Thus, Applicants provide all the information and more necessary for one skilled in the art to practice the subject matter of the claims without undue experimentation.

Therefore, Applicants' claims are enabled by the specification for at least the following reasons: they are commensurate in scope with the specification, the level of predictability is such that candidate residues for substitution with bulky amino acids are readily identified and the resulting functional/structural change foreseen, and the amount of direction provided by the inventors is sufficient to enable one of ordinary skill to practice the claimed subject matter without undue experimentation.

III. Claim Rejections under 35 U.S.C. § 102(b)

The Examiner asserts claims 1, 27-29, and 31-45 are anticipated by Dillon *et al.* (U.S. 2007/0009942) under 35 U.S.C. § 102(b). In order for a reference to be a statutory bar, it must patent or describe the invention in a printed publication more than one year prior to the date of the application for patent in the United States. 35 U.S.C. § 102(b). If the application properly claims benefit under 35 U.S.C. § 119(e) to a provisional application, the effective filing date is the filing date of the provisional application for any claims which are fully supported under the first paragraph of 35 U.S.C. § 112 by the provisional application. MPEP § 706.02 V (D). Here, the present application claims priority to Provisional Application Serial No. 60/464,081 filed on April 18, 2003, just 6 days after the filing date and 18 months prior to the publication date of the Dillon *et al.* priority application (U.S. 7,135,290). Thus, Dillon *et al.* is not an appropriate § 102(b) reference.

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For the reasons set forth above, Applicants respectfully submit the claims are allowable and reconsideration and issuance of a notice of allowance are respectfully requested. If it would be helpful to obtain favorable consideration of this case, the Examiner is encouraged to call and discuss this case with the undersigned.

This constitutes a request for any needed extension of time and an authorization to charge all fees therefore to deposit account No. 14-0460 if not otherwise specifically requested. The undersigned hereby authorizes the charge of any required fees not included or any deficiency of fees submitted herewith to be charged to deposit account No. 14-0460.

Respectfully submitted,

Date: 1/10/08

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